BIBAC at appropriate locations within the heterologous DNA. For example, a 56 kb gene cluster responsible for synthesis of the polyketide epothilone (a potential anticancer agent) in the myxobacterium *Sorangium cellulosum* was cloned into two plasmids for heterologous expression in *Streptomyces coelicolor* (Tang et al., 2000). This cluster could have been cloned as one segment into the BIBAC, with incorporation of appropriate promoters sequences for expression in a chosen host.

[0029] For screening of libraries and DNAs isolated from nature for production or degradation of chemicals and products, it is advantageous to look for such phenotypes without further engineering of the heterologous DNA for gene expression. Such engineering requires time-consuming sequencing and characterization of the heterologous DNA, as described in the preceding paragraph for the epothilone pathway. As an alternative to further engineering, hosts containing the BIBAC can be screened for products synthesized by expression of the heterologous DNA from endogenous promoters that happen to be expressed in the host. It is not uncommon for bacterial promoters from one species to be recognized and the gene expressed in other bacterial species. Such recognition is thought to contribute to horizontal gene transfer between species (Davison, 1999; Kroll et al., 1998; Lawrence, 1999). As an example of natural heterologous expression, a DNA fragment of *Agrobacterium tumefaciens* conferred the ability to metabolize sucrose on *E. coli* strains (Schuerman et al., 1997). Heterologous expression is not limited to bacterial species; eukaryotic DNA may sometimes be expressed in bacterial host organisms. For example, it has been shown that plant viral and plant promoters can be expressed in *E. coli, Yersinia enterocolitica* and *Agrobacterium tumefaciens* (Lewin et al., 1998).

[0030] As an alternative to utilizing recognition of heterologous gene regulatory sequences by endogenous host factors, enhanced expression may be achieved by expressing genes for

regulatory factors from the source organism in the BIBAC host. For example, enhanced expression of bacterial genes when the BIBAC is incorporated into yeast may be obtained if the RNA polymerase and/or sigma-like factors from the bacterial source of the heterologous DNA are co-expressed in yeast. Such regulatory factor genes could be incorporated into the BIBAC vector or into a second vector or into the chromosomal DNA of the host organism.

[0031] The method of the present invention can be used in a number of applications. One application is screening a genomic library for expression of a desired gene product. In this method, genomic DNA from a donor can be cut with a restriction endonuclease (BamHI in the case of the BIBAC vector). The restriction fragments which represent collectively the entire genome of the donor are then each ligated into a vector (which has been opened by cutting with BamHI in the case of the BIBAC vector). This generates a library in the vector. (See generally, Current Protocols in Molecular Cloning, Ausubel, F.M. et al., eds., Greene Publishing and Wiley Interscience, New York (1989).) Alternatively, DNA can be obtained by performing PCR or other gene amplification methods from DNA purified from natural sources, including DNA of non-culturable species. Donor organisms can be any organism such as prokaryotes, animals, plants, and DNA from unknown non-culturable microorganisms.

[0032] For ease in working with the library, the vector is generally maintained in a bacterial host cell. *Escherichia coli* is a standard bacterial host cell for maintaining such a DNA library. The vector DNA can be introduced into the bacterial host cell by various methods known in the art. These include electroporation, calcium chloride transformation, and transformation by particle bombardment. The transformed bacterial cells can be identified by their ability to grow on various selective agents. Accordingly, bacterial cells that contain the vector can be identified by their resistance to kanamycin. The presence of inserted heterologous DNA is indicated by the

ability of the bacteria to grow on high levels of sucrose. The ability to grow on high levels of sucrose is due to the inactivation of another selection marker, the *sacB* gene. Potential clones containing the desired heterologous DNA can be identified by Southern analysis (Southern 1975) using closely linked molecular markers or heterologous DNA as probe, *in situ* hybridization assays; or PCR probes for detecting sequences related to polypeptides encoded by the heterologous DNA in the vector; oligolabeling, nick translation, end-labeling or PCR amplification using a labeled nucleotide. Clones (vector containing heterologous DNA) of interest are then used for further experiments. The bacterial host containing the clone can be screened for expression of a desired protein or synthesis or degradation of a chemical by methods well known in the art. For example, using either polyclonal or monoclonal antibodies directed against the desired protein, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), fluorescent activated cell sorting (FACS), SDS-PAGE gels, Western blots, etc.

[0033] If desired, the vector, or a derivative clone of interest (as described above), can be introduced into another prokaryotic host. This introduction can be accomplished using methods known to those in the art, including electroporation, or particle bombardment. Another method that can be used to introduce the vector into certain species such as *Agrobacterium tumefaciens* is triparental mating. In a triparental mating, the *Escherichia coli* containing the vector, a second *Escherichia coli* containing a helper plasmid, and an *Agrobacterium* are combined, resulting in introduction of the vector DNA into the *Agrobacterium*. The *Agrobacterium* cells are then screened using a selection marker (such as kanamycin resistance in the BIBAC vector), for the presence of the vector DNA therein. Those cells containing the vector DNA are then used for further experiments, and/or screened for expression of a desired gene product.